TEMPERATURE RELATIONS OF AERIAL AND AQUATIC RESPIRATION IN SIX LITTORAL SNAILS IN RELATION TO THEIR VERTICAL ZONATION [1]

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All larger intertidal animals are alternately aerial and aquatic in habit. The majority of littoral molluscs (surprisingly including bivalves as well as gastropods) are adapted for respiration both as air-breathers and by aquatic ventilation of ctenidia or other "gill" structures. Temperature relations of aquatic respiration in one of the most common littoral snails, *Littorina littorea* have been extensively investigated (Newell, 1969, 1973; Newell and Pye, 1970a, b, 1971a, b; Pye and Newell, 1973; Newell and Roy, 1973), and the occurrence of some degree of regulation in "standard metabolism" has been established for that species (Newell and Pye, 1970b; Pye and Newell, 1973). Comparative studies on congeneric and other related intertidal snails are less extensive (Sandison, 1966, 1967; Toulmond, 1967a, b). The present report deals with temperature-induced shifts in oxygen uptake rates for the aquatic respiration of six (and the aerial respiration of four) species of littoral and sublittoral snails. Parallel studies (McMahon and Russell-Hunter, 1974, 1977) on responses to low oxygen stress revealed considerable interspecific differences, but these were less related to vertical zonation on seashores than to microenvironmental factors and to the physiological adaptations of each individual species. With interspecific (and even with infraspecific) differences in temperature relations, we are somewhat more directly concerned with degrees of aerial exposure and hence with the principal patterns of their distributional ecology including intertidal vertical zonation.

Using oxygen electrodes, respiration rates in sea water over a range of natural temperatures were determined for *Acmaea testudinalis, Mitrella lunata, Lacuna vincta, Littorina obtusata, L. littorea,* and *L. saxatilis.* Corresponding aerial respiration rates were determined for *Acmaea* and for the three *Littorina* spp. Early results in these investigations were reported in two preliminary abstracts (McMahon and Russell-Hunter, 1973; Russell-Hunter and McMahon, 1974).

MATERIALS AND METHODS

Determinations of oxygen uptake rates were made for six species of marine prosobranch gastropods: three sublittoral species, *Lacuna vincta, Mitrella lunata,* and *Acmaea testudinalis;* and three intertidal species, *Littorina obtusata, Littorina*

littorea, and *Littorina saxatilis.* Current American usage is adopted in the nomenclature of the three common *Littorina* spp., *L. obtusata, L. littorea,* and *L. saxatilis.* The "Smooth Periwinkle" of lower levels in the intertidal zone, usually associated with fucoid seaweeds including *Ascophyllum,* is called *Littorina obtusata.* This periwinkle is referred to in modern European physiological literature as *L. littoralis* (L.) (not to be confused with the distinct species *L. littorea* which also occurs in Europe), and in early U. S. conchological literature as *L. palliata* (Say). The "Common Periwinkle" of midlevel intertidal, on most temperate Atlantic shores the largest, most common, and most abundant periwinkle, is universally known as *Littorina littorea.* At higher levels of the intertidal zone around MHWNT is the "Rough Periwinkle" of variable shell form and color, which is here called *Littorina saxatilis.* In most literature, both American and European, *L. rudis* is simply a synonym of *L. saxatilis,* but future studies may divide this polymorphic species.

Lacuna vincta, M. lunata and the three *Littorina* spp. were collected from a boulder beach on Nobska Point (41° 30.9′ N; 70° 39.3′ W) near Woods Hole, Massachusetts on the southern shore of Cape Cod. *A. testudinalis,* along with *Lacuna vincta* and the same three *Littorina* spp., were collected from a boulder beach at Manomet Point, Massachusetts (41° 55.2′ N; 70° 31.2′ W) in the colder waters just north of Cape Cod. During the collection period in the summer of 1973, surface water temperatures ranged from 17.5° C to 25° C at Nobska Point (July 18 through August 12, 1973), giving a mean of 21.6° C, while over the same period mean temperature at Manomet Point was 17.4° C (range = 17.0°–18.0° C).

After each species' collection, the snails were brought to the laboratory in an insulated jar and maintained at ambient water temperature in an incubator in 100% (31.0‰) sea water. All oxygen uptake rate determinations were begun within 72 hours of the time of collection. Oxygen uptake rates in water and in air were monitored with Clark-type polarographic oxygen electrodes (Clark, 1956), purchased from Yellow Springs Instrument Company (Model-53). Respiration chambers were maintained at constant temperatures (± 0.05° C) with a Haake-type Fe constant temperature circulator. The glass respiration chambers (6.9 cm high, 2.04 cm internal diameter) were modified with a glass annulus to contain snails under the magnetic stirrer (diagrams of this and similar modifications are given in Burky, 1977; for use see also: Burky, 1969, 1971; McMahon, 1972, 1973; McMahon and Russell-Hunter, 1977). With this modification the magnetic stirrer spins at approximately 180 revolutions per minute above the snails, and this allows adequate mixing of water in the chamber. The intertidal snails of this study all came from shores which receive moderate amounts of wave action. All six species studied have an adhesive foot and were able to remain actively crawling while attached to the chamber floor and to the walls of the annulus. The currents of the chamber perhaps simulate the water movements experienced by these species in their natural environment. Changes in chamber oxygen concentration were continuously recorded on a Honeywell Electronik-16 Strip Chart Recorder. Aquatic oxygen consumption determinations were made for four to eight subsamples, each of one to ten individuals for the six species studied. The class interval for each subsample was ± 2.5 mm around a chosen shell height, and subsamples were selected to represent the range of shell heights encountered in each field population.

Each experimental subsample was placed in a respiration chamber at ambient field temperature with either 4 or 5 ml of previously boiled and filtered sea water (salinity = 31.0‰, chlorinity = 17.71‰). Temperature was then lowered 3° C every five minutes until 5° C was reached. After equilibration in a blank chamber (with sea water but without snails), the oxygen probe was seated in an experimental chamber and oxygen uptake was continuously monitored from full air saturation ($Po_2 = 159.1$ mm Hg) to an oxygen tension of 90%, or monitored over the reduction in oxygen tension from full air saturation after 30 minutes. This procedure was repeated for all experimental subsamples at 5° C intervals from 5° C through the upper lethal temperature, which varied from 35° C to 45° C depending on species.

During the summer of 1974, oxygen uptake rates from air were determined for *A. testudinalis* from Manomet Point and *L. obtusata, L. littorea* and *L. saxatilis* from Nobska Point. In order to determine aerial respiration rates, the oxygen electrode probe was modified with a neoprene O-ring forming an airtight seal with the chamber wall and by a short length of plastic tubing (1 mm external diameter) to form an air vent to the chamber. The oxygen probe could be inserted in the glass chambers to enclose a sealed volume of 2, 4 or 6 ml of air. (For each species, this volume was less than the corresponding water volume used for measurements of aquatic oxygen uptake.) Aerial oxygen uptake rates were determined for four subsamples of *A. testudinalis*, and for six subsamples each of the three species of *Littorina*. Each subsample contained one to eight individuals depending on size. Snails were blotted on filter paper and placed into the respiration chambers at room temperature (21–23° C). Chamber temperature was then lowered at a rate of 3° C every five minutes until a chamber temperature of 5° C was reached. The oxygen electrode was then equilibrated for 25 minutes in a blank chamber,

TABLE I

Standard weights and corresponding shell heights for the "standard" adult snail in the six intertidal species used. Oxygen uptake values for experimental series (Figures 1-5) were computed by applying these weight values to the 120 regressions of dry tissue weight against oxygen uptake rate, at the appropriate temperatures.

Species	Population	Standard dry tissue weight mg	Corresponding average shell height mm	Number of determinations	Number of regressions	Graphs using this standard
Acmaea testudinalis (Müller)	Manomet	8.3	11.0	90	18	Figs. 1, 2
Mitrella lunata (Say)	Nobska	0.92	4.0	36	7	Fig. 1
Lacuna vincta (Montagu)	Manomet	1.2	3.8	36	7	Fig. 1
	Nobska	1.2	4.3	36	7	Fig. 1
Littorina obtusata (L.)	Manomet	10.0	6.5	72	9	Fig. 3
	Nobska	10.0	7.6	126	18	Fig. 3
Littorina littorea (L.)	Manomet	82.0	14.6	72	9	Fig. 4
	Nobska	82.0	15.4	126	18	Fig. 4
Littorina saxatilis (Olivi)	Manomet	7.8	5.5	72	9	Fig. 5
	Nobska	7.8	6.5	126	18	Fig. 5

before being placed in an experimental chamber, and allowed to equilibate a further 10 minutes with the vent tube open. The vent tube was then clamped shut, and oxygen consumption was then monitored as has been described earlier for aquatic respiration. This procedure was then repeated for all experimental groups at 5° C intervals from 5° C, until each specific upper lethal temperatures was reached. A small piece of silica gel desiccant was placed in both the experimental and blank chambers to obviate water condensation.

After aerial oxygen uptake determinations were complete, the snails in each subsample were blotted on filter paper and their volume and that of the silica gel granule measured by fluid displacement. Each volume of snails plus silica gel was then subtracted from the appropriate chamber volume to give the air volume for that experiment.

After both aquatic and aerial oxygen uptake experiments, the calcareous shells were then removed by treatment with 5% nitric acid for 6 hours. The remaining tissue was rinsed in distilled water and dried to constant weight (95° C for over 12 hours). For each species subsample, oxygen uptake rates were computed as microliters of oxygen consumed per milligram shell-free dry tissue weight per hour [μl $O_2/(mg \cdot hr)$] at STP. Logarithmic regressions of dry tissue weight *versus* oxygen uptake rate in water and in air at each experimental temperature

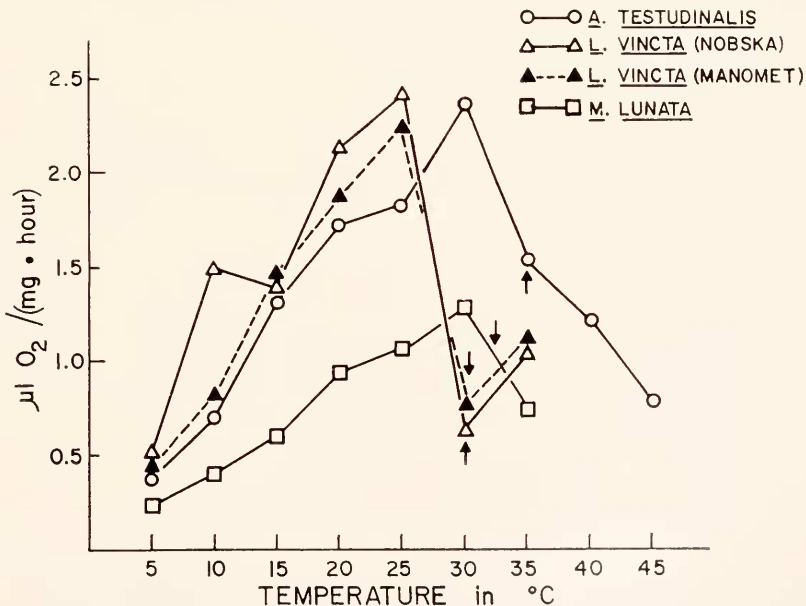FIGURE 1. The effect of temperature on aquatic oxygen uptake rates in *Acmaea testudinalis, Lacuna vincta* and *Mitrella lunata*. The vertical axis represents oxygen uptake rate in microliters of oxygen per milligram shelless dry tissue weight per hour; and the horizontal axis, temperature in degrees centigrade. The open circles represent the oxygen uptake rates of *A. testudinalis* collected from Manomet Point, Massachusetts; the open triangles, that of *L. vincta* from Nobska Point, Massachusetts; the solid triangles, that of *L. vincta* from Manomet Point; and the open squares, that of *M. lunata* from Nobska Point. The vertical arrows indicate approximate lethal temperatures for each experimental group.

were then computed for each species-population tested. (The 120 regression equations are not set out here but can be made available by the senior author, along with the raw data, to any interested investigator.) A species oxygen consumption rate for each experimental temperature was then estimated from these regressions for a standard individual with tissue dry weight representing a modal (median weight) value for a mature adult of that species-population (Table I).

RESULTS

All three subtidal species studied, *Acmaea testudinalis, Lacuna vincta* and *Mitrella lunata,* had similar patterns of respiratory response to increasing temperature. Aquatic oxygen uptake rates in all three species increased steadily from low values at 5° C to peak uptake rates at 25–30° C, which were then immediately followed by a marked decrease in respiration rate at 30–35° C due to thermal stress leading to death (Fig. 1). The Q_{10} of oxygen uptake was very similar for all three species, with *A. testudinalis* having a Q_{10} of 2.05 over 5–30° C. For *M. lunata* Q_{10} was 2.01 over 5–30° C, and for *L. vincta* Q_{10} was 2.15 over 5–25° C for the Nobska Point population, and 2.22 over 5–25° C for those from Manomet Point. Oxygen uptake rates between 5° and 25° C were similar in *A. testudinalis* and *L. vincta,* while *M. lunata* had oxygen rates which were about 50% less up to 25° C (Fig. 1). *L. vincta* from the colder Manomet Point environment (see
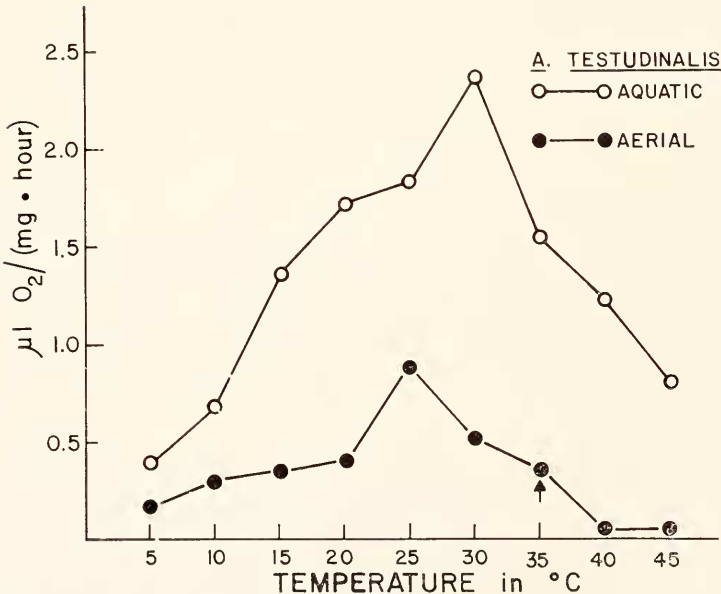

FIGURE 2. The effect of temperature on the aquatic and aerial oxygen uptake rates of *Acmaea testudinalis.* The vertical axis represents oxygen uptake rate in microliters of oxygen per milligram shelless dry tissue weight per hour; and the horizontal axis, temperature in degrees centigrade. The open circles represent the aquatic oxygen uptake rates; and the solid circles, the aerial oxygen uptake rates of *A. testudinalis* collected from Manomet Point. The vertical arrow indicates the approximate lethal temperature.
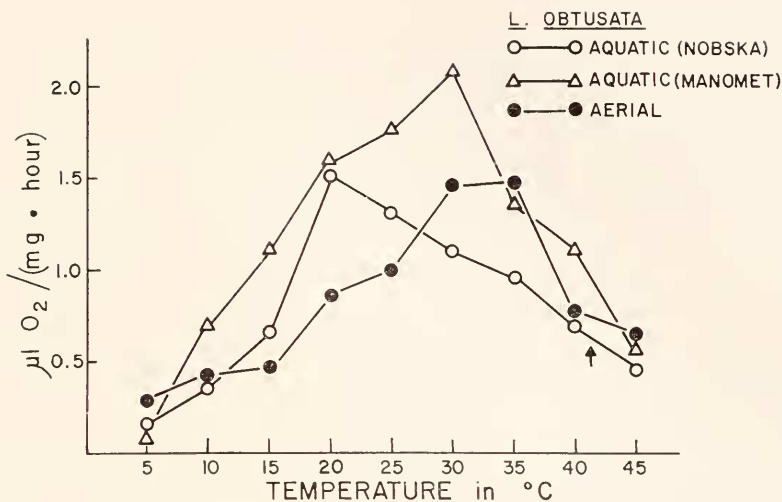
FIGURE 3. The effect of temperature on the aquatic and aerial oxygen uptake rates of *Littorina obtusata*. The vertical axis represents oxygen uptake rate in microliters of oxygen per milligram shelless dry tissue weight per hour; and the horizontal axis, temperature in degrees centigrade. The open circles represent the aquatic oxygen uptake rates; and the solid circles, the aerial oxygen uptake rates of *L. obtusata* collected from Nobska Point. The open triangles represent the aquatic oxygen uptake rates of *L. obtusata* from Manomet Point. The vertical arrow indicates the approximate lethal temperature.

Methods) had a respiratory rate which averaged 12.7% less than that for specimens from Nobska Point through 25° C. Thermal death occurred at about 30° C in *L. vincta* from both the Manomet and Nobska Point populations, at 32.5° C in *M. lunata* and at 35° C in *A. testudinalis* (Fig. 2).

The effects of temperature on the oxygen uptake of *A. testudinalis* in air are similar to those which occur in aquatic respiration. However, oxygen consumption in air is markedly reduced from aquatic rates at all temperatures (Fig. 2) and may reflect the large quantity of mucus secreted over the body surface on exposure of these limpets to air. In air the oxygen consumption of *A. testudinalis* increases from a low rate at 5° C to a peak consumption rate at 25° C. However, the Q_{10} of aerial oxygen uptake is very similar to that recorded for aquatic respiration at 2.20 (Fig. 2). Between 5° and 35° C, aerial oxygen consumption in *A. testudinalis* is only 36.4% of (or 2.75 times less than) aquatic oxygen uptake rates over the same temperature span (Fig. 2).

The patterns of oxygen uptake with increasing temperature in the three congeners, *Littorina obtusata*, *L. littorea* and *L. saxatilis*, were markedly different from those of the three subtidal species, with each of the three species displaying a uniquely characteristic respiratory response to increasing temperature (Figs. 3–5).

L. obtusata shows a steady increase in aquatic oxygen consumption rates from 5° C up to 20° C in the Nobska Point population, and up to 30° C in the Manomet Point population. The corresponding Q_{10} values are 4.42 (5°–20° C) for the specimens from Nobska Point and 3.18 (5°–30° C) for those from Manomet Point (Fig. 3). Unlike the three sublittoral species, decreases in aquatic oxygen

consumption rates from their peak values are not associated with immediate thermal death in *L. obtusata*. Instead, aquatic oxygen uptake rates decrease steadily from either 20° C or 30° C with increasing temperatures until death ensues at about 41.5° C (Fig. 3). The oxygen consumption for specimens of *L. obtusata* from Manomet Point was 47.2% greater than that of specimens from the warmer Nobska Point environment over a temperature range of 5° to 40° C (Fig. 3).

The aerial oxygen uptake in *L. obtusata* from Nobska Point showed a steady increase from 5° to 35° C (Fig. 3). Rates of oxygen consumption in air were similar to, or lower than, aquatic rates for Nobska Point snails between 5°–25° C and higher than aquatic oxygen uptake rates at 30° and 35° C. At 40° C oxygen uptake rates from air were nearly equal to those from water (Fig. 3). For *L. obtusata*, the Q_{10} of oxygen uptake in air was 1.72 over 5°–35° C, a value much lower than the Q_{10} of aquatic oxygen uptake recorded from either the Nobska or Manomet Point populations.

The oxygen uptake of *Littorina littorea* from both water and air was characterized by the maintenance of lower oxygen consumption rates than the other species at all experimental temperatures, and also by an apparent regulation of respiratory rate between 20° and 30° C (Fig. 4). Aquatic oxygen consumption increased from 5° to 20° C in *L. littorea* from Manomet Point with a Q_{10} of 3.63, and from Nobska Point with a Q_{10} of 3.66. However, in both groups, aquatic oxygen uptake showed very little change in rate between 20° and 30° C, with Q_{10} values of 1.04 (Manomet Point) and 0.95 (Nobska Point). At temperatures higher than 35° C, aquatic oxygen consumption decreased steadily in both Nobska and Manomet snails until death occurred at about 43° C. Aquatic rates were very similar in both the Nobska and Manomet Point *L. littorea* populations, with the oxygen uptake rate of Nobska animals an average of only 2.6% greater than that of Manomet animals from 5° to 40° C.
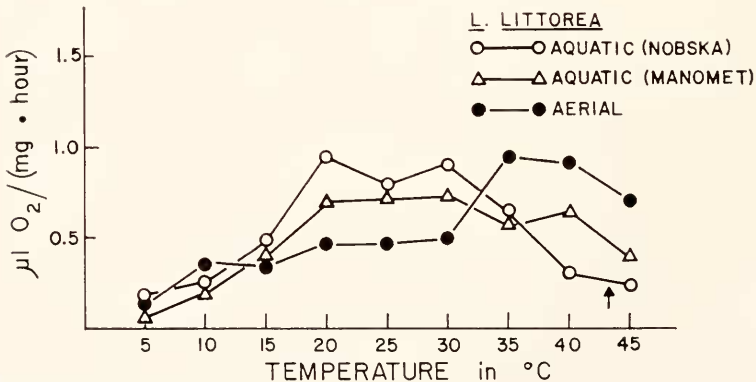

FIGURE 4. The effect of temperature on the aquatic and aerial oxygen uptake rates of *Littorina littorea*. The vertical axis represents oxygen uptake rate in microliters of oxygen per milligram shelless dry tissue weight per hour; and the horizontal axis, temperature in degrees centigrade. The open circles represent the aquatic oxygen uptake rates; and the solid circles, the aerial oxygen uptake rates of *L. littorea* collected from Nobska Point. The open triangles represent the aquatic oxygen uptake rates of *L. littorea* from Manomet Point. The vertical arrow indicates the approximate lethal temperature.
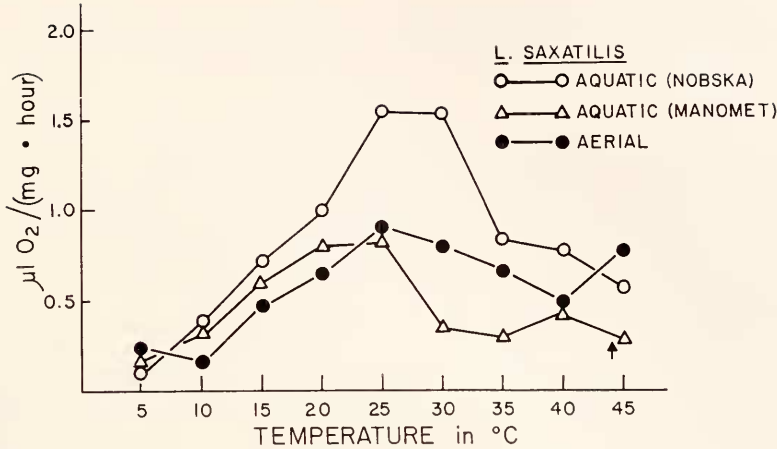
FIGURE 5. The effect of temperature on the aquatic and aerial oxygen uptake rates of *Littorina saxatilis*. The vertical axis represents oxygen uptake rate in microliters of oxygen per milligram shelless dry tissue weight per hour; and the horizontal axis, temperature in degrees centigrade. The open circles represent the aquatic oxygen uptake rates; and the solid circles, the aerial oxygen uptake rates of *L. saxatilis* collected from Nobska Point. The open triangles represent the aquatic oxygen uptake rates of *L. saxatilis* from Manomet Point. The vertical arrow indicates the approximate lethal temperature.

The aerial oxygen uptake of *L. littorea* from Nobska Point showed a similar area of regulation between 20° and 30° C with a Q_{10} of only 1.06. Aerial respiration rate increased between 5° and 20° C with a Q_{10} of 2.68. Unlike aquatic respiration, the aerial oxygen uptake rate of *L. littorea* increased at temperatures higher than 30° C and remained elevated, until death due to thermal stress occurred at about 43° C. In *L. littorea* from Nobska Point, aquatic uptake rates were 47.9% greater between 5° and 30° C than aerial rates, while at 35°–40° C, aerial rates were an average of 125% greater than aquatic rates (Fig. 4).

Littorina saxatilis showed aquatic oxygen uptake rates which increased from 5° to 25° C, with corresponding Q_{10} values of 3.01 (Nobska Point) and 2.49 (Manomet Point) (Fig. 5). Aquatic rates declined sharply at temperatures higher than either 25° or 30° C and remained relatively unchanged, until death occurred at about 44° C. Thus, *L. saxatilis* from Nobska Point had a Q_{10} of 0.85 from 35°–40° C, and Manomet Point animals had a similar Q_{10} of 1.24 over 30°–40° C (Fig. 5). It is of interest to note that the rate decline occurred at 25° C for snails from the cooler Manomet Point habitat, while it occurred at 30° C in *L. saxatilis* from the warmer Nobska Point habitat. At all experimental temperatures, *L. saxatilis* from Nobska Point had uptake rates averaging double those for Manomet Point snails.

Aerial oxygen consumption rates for *Littorina saxatilis* from Nobska Point were generally lower than aquatic rates. However, they showed a pattern of response to increasing temperature which was similar to that for aquatic respiration. In air, rates increased from 5° to 25° C with a Q_{10} of 1.93. At temperatures above 25° C, aerial oxygen consumption decreased continuously until the onset of thermal

death at $44°$ C ($Q_{10} = 0.59$, from $30°–40°$ C) (Fig. 5). For *L. saxatilis* from Nobska Point, aquatic oxygen consumption rates averaged 57.8% greater than aerial respiration rates from $5°$ to $25°$ C.

DISCUSSION

The investigation reported in this paper differs from earlier studies on the respiration of intertidal snails in two respects. First, it presents measurements of oxygen uptake made on normally active snails, unlike earlier studies involving a somewhat arbitrarily defined "standard metabolism." Secondly, it is comparative and provides equivalent measures of aquatic oxygen uptake for six, and of aerial uptake for four, different species which live in different vertical zones of the littoral, in contrast to the best of earlier studies (Newell, 1969, 1973; Newell and Pye, 1970a, b, 1971a, b; Pye and Newell, 1973; Newell and Roy, 1973) which are largely limited to one species, *Littorina littorea*. This discussion will briefly consider the relationship between "basal," "standard" and "active" respiratory rates in molluscs such as intertidal snails, before passing to a more extensive consideration of the relationships between patterns of respiratory response to temperature in each species and their distributional ecology, including intertidal vertical zonation.

For many poikilothermic invertebrates, including the majority of littoral and freshwater gastropods, there is no "basal" metabolic rate as the term is understood in vertebrate physiology. In such snails, rates, patterns, and even modes of oxygen uptake are extremely plastic (Russell-Hunter, 1964, 1977). In the earlier work by Newell and his associates (see, for definitions, Newell, 1966; Newell and Pye, 1970b), the difficulty of rates of oxygen uptake varying with degrees of activity was dealt with in the following manner. Regression values of rates against tissue dry weights (least-squares fitting) yielded maximal and minimal curves, which were then termed the "active" rate and the "standard" rate, respectively, and permitted division of the data. In *Littorina littorea*, a zone of thermoregulation of oxygen uptake could only be demonstrated in the minimal or "standard" rate (Newell and Pye, 1970a, b). Subsequently, more sophisticated computations (Newell and Roy, 1973) showed that similar thermoregulation could be claimed for the "active" rates. Of course, many systems both of pallial ciliation and buccal musculature are continuously active in all such gastropods and, under certain conditions in the field, slow locomotion and radular grazing are continuous in many of them. Minimal rates must always be suspect and can only dubiously be claimed as "standard." The investigation reported here concerned normally active snails, and there was no arbitrary division of the data into minimal and maximal groupings; *all* data were utilized in the computation of uptake rates. Snails were chosen to be representative of the size classes (cohorts) present in the natural populations.

Partly as a result of these methodological differences, the work of Newell and his associates is moving towards a more complete elucidation (Newell and Pye, 1970a, b; Newell, 1973) of the cellular and enzymatic bases for respiratory adjustment; while the work reported here (and also on low oxygen stress in the same snails, McMahon and Russell-Hunter, 1974, 1977) allows consideration of interspecific differences and ecological relationships with respiratory measures more closely approximating those for naturally active snails in field populations.

Before the present work, comparative studies on congeneric and other related
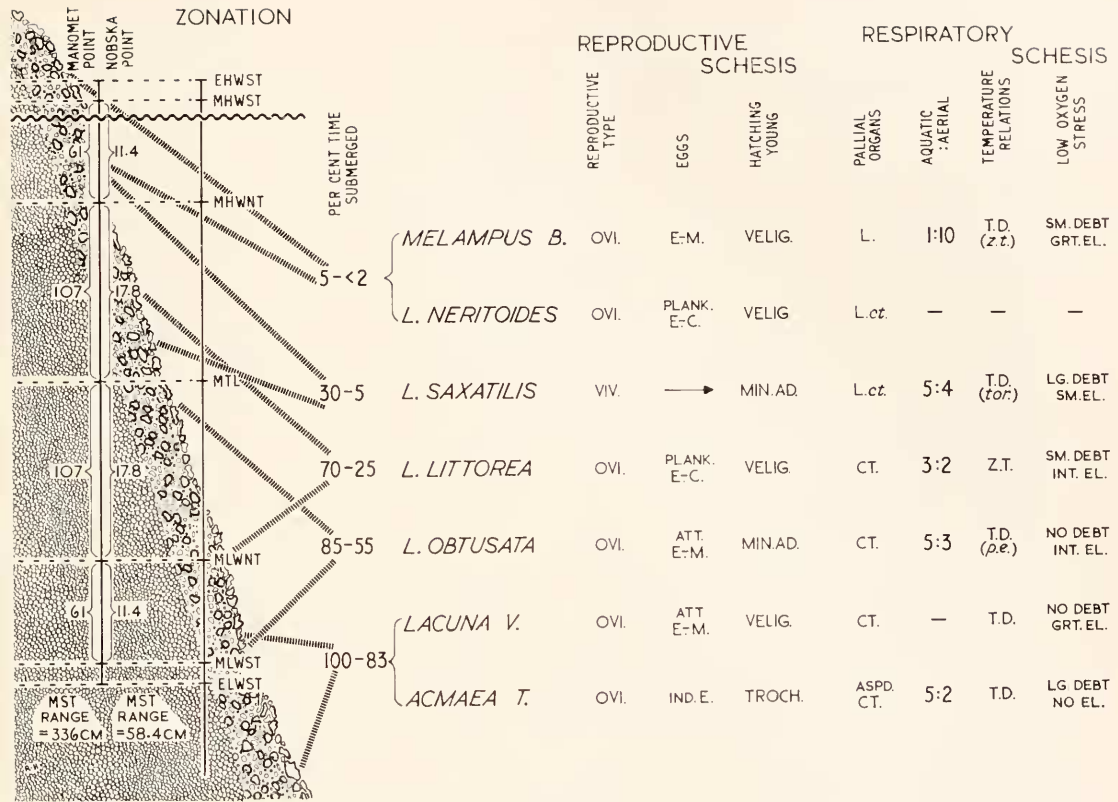
FIGURE 6. Vertical zonation in the intertidal with reproductive and respiratory scheses of seven species of littoral snails. Average percentages of time spent submerged are indicated, along with the actual mean spring tide (MST) ranges in centimeters at both Manomet Point and Nobska Point, Massachusetts, U. S. A. The seven species are *Melampus bidentatus, Littorina neritoides, Littorina saxatilis, Littorina littorea, Littorina obtusata, Lacuna vincta,* and *Acmaea testudinalis.* In the three columns concerned with reproduction, the basic reproductive type is indicated as oviparous (OVI.) or viviparous (VIV.); the egg-laying pattern as individual eggs (IND.E.), egg-capsules (E.-C), or egg-masses (E.-M.) which may be attached (ATT.) or planktonic (PLANK.); and the hatching young as trochophore larvae (TROCH.), veliger larvae (VELIG.) or with the body form of miniature adults (MIN. AD.). In the four columns to the right concerned with respiration, the pallial respiratory organs are designated as having the typical prosobranch gill or pectinibranch ctenidium (CT.), the more primitive "feather-gill" or aspidobranch ctenidium (ASPD. CT.), the mantle-cavity as an air-breathing lung (L.), or as a lung with a vestigial ctenidium (L. ct.); the average ratio of aquatic to to aerial oxygen consumption (both assessed at about 22° C) is shown; temperature relations (similar for both aquatic and aerial respiration) are indicated as showing a zone of thermoregulation (Z.T.) or as being largely temperature dependent (T.D.), the latter being modified by some thermoregulation at higher temperatures [T.D. (z.t.)], by torpor at higher temperatures [T.D. (tor.)], or by involving passive endurance [T.D. (p.e.)]; and lastly, the effects of low oxygen stress involve payment of a large (LG.), small (SM.), or NO oxygen debt, and great (GRT.), intermediate (INT.), small (SM.), or NO elevation of post-stressed rates of oxygen uptake at lower (10%) oxygen concentrations. Note that no new data are presented for *Littorina neritoides,* which does *not* occur in our study areas on the mid-Atlantic coast of North America. Although the general adaptational trends from *Acmaea* to *Melampus* are from sea to land, it is clear that the evolution of near-terrestrial structures and functions has proceeded anacoluthically. For further discussion, see text.

intertidal snails were less extensive. Sandison (1966, 1967), and Toulmond (1967a, b) each investigated oxygen consumption in several littoral snails, along the lines of the earlier work of Raffy (1933, see also Fischer, Duval, and Raffy, 1933). Toulmond (1967a, b) worked at a single temperature, and demonstrated that aerial respiration is similar to aquatic in *L. littorea,* close in *L. saxatilis,* but is markedly greater in *L. obtusata.* The earlier French work had reported that aquatic respiration in *L. neritoides* and *L. saxatilis* was several times greater than aerial respiration. Sandison (1966, 1967) claimed that aerial respiration was significantly greater than aquatic respiration in all three species of *Littorina* over a temperature range from 18° C to thermal death (37°–41° C). Obviously, there are contradictory data on the relationship between aquatic and aerial respiration. The results of Raffy and of Toulmond (except for *L. obtusata*) are in agreement with those of the present paper but contradict Sandison's claims. Other earlier reports include excellent comparative studies on desiccation resistance, salinity tolerances, temperature stresses, and levels of thermal death in several series of littoral gastropods in relation to vertical zonation, but do not present any adequate data on respiration rates (Colgan, 1910; Gowanloch and Hayes, 1926; Broekhuysen, 1940; Evans, 1948; North, 1954; Southward, 1958a; Fraenkel, 1960, 1961, 1966, 1968; Lewis, 1963; Markel, 1971).

We have been principally concerned with establishing, for six species of intertidal snails, the patterns of response to temperature change in rates of oxygen uptake. These patterns *do* reflect littoral zonation, particularly the vertical distribution of each species. Three, *Acmaea testudinalis, Lacuna vincta* and *Mitrella lunata,* are essentially sublittoral species which stray into the lower levels of the true littoral and show similar patterns of respiratory response. Aquatic oxygen uptake increases at a constant rate up to 25° or 30° C, and this is followed by a steep decline in uptake leading to thermal death at around 35° C. Aerial uptake, assessed only for *Acmaea,* is lower (between 30% and 50% of aquatic uptake at the same temperatures) but follows the same pattern. In these three species (and probably in the majority of snail species living subtidally), there is no obvious metabolic adjustment to higher temperatures. The other species, *Littorina obtusata, L. littorea* and *L. saxatilis,* are true intertidal snails and, despite some overlaps, the three congeners show a clear vertical zonation. In *L. obtusata,* the "lowest" of the three, both aerial and aquatic uptake rates increase steadily within the normal temperature range (up to about 25° C), then decline slowly (in a "passive endurance," quite unlike the responses of the sublittoral species). In the dominant snail of the midlittoral, *Littorina littorea,* aerial and aquatic respiration are rather similar up to 30° C (corresponding to normal upper limits for their environment). Unlike *Acmaea* and *L. obtusata,* increase in uptake for *L. littorea* between 15° C and 30° C is considerably less than between 5° C and 15° C (or, for that matter, above 30° C), constituting a zone of metabolic regulation, in both aerial and aquatic respiration. Finally, *Littorina saxatilis* shows somewhat lower aerial than aquatic respiration rates at all temperatures. The lower rates for *L. saxatilis* above 25° C occur well below temperature levels at which thermal deterioration sets in. These lower rates represent a reversible "torpor" or short-term diapause, which fits with the specific habitat (70%–95% of their time out of water). It is worth noting that these major shifts, with changes of temperature, and between aerial and aquatic respiration, are many times greater than the rhythmic metabolic shifts

known to occur under near-constant conditions (see, for example, Sandeen, Stephens and Brown, 1954).

In discussing the ecological significance of these respiratory patterns, it is appropriate to assess for each level of habitat the mean proportion of time in each semi-lunar tidal cycle of 354.4 hours when that particular zone is actually bathed by sea water. In Figure 6 are shown the average percentages of time spent submerged, as well as the vertical distributions of the species and the actual tidal ranges involved at Nobska and Manomet Points. *Mitrella lunata*, being essentially similar to *Acmaea* and *Lacuna* is omitted from Figure 6, while two species of the highest levels of the littoral are included for comparison: a fourth congener, *Littorina neritoides* (which does *not* occur on the mid-Atlantic coasts of North America, and for which we have no new data), and the primitive pulmonate of high-level salt-marshes, *Melampus bidentatus* (for which we have some respiratory and survivorship data, McMahon and Russell-Hunter, 1975). Figure 6 also presents an attempt to summarize certain significant aspects of both the reproductive and respiratory scheses of these seven snails. Taken as a whole, the general adaptational trends run from sea to land—from *Acmaea* to *Melampus*—with many irregularities intervening in each functional series. With the obvious exception of the serial shift from an aspidobranch ctenidium (a gill suitable only for clean waters) to a lung, the evolution of near-terrestrial structures and functions has proceeded anacoluthically. Similarly discontinuous series (not presented here) would involve water-control and nitrogenous excretion in these snails. Although doubts have arisen regarding the much-quoted figures on the uric content of gastropods which were first estimated by Needham (1935, 1938; see also Spitzer, 1937; Campbell, 1973), retention of uric acid (though perhaps not of a predominantly uricotelic metabolism paralleling that of reptiles and birds) is apparently greater in *Littorina neritoides* and *L. saxatilis* than it is in *L. obtusata*, while it is least in *L. littorea*. The "higher" species of *Littorina* have a greater capacity to resist water-loss but, under desiccating conditions, rates of body water loss in *Melampus bidentatus* are drastically high. This species is characterized not only by tissues extremely tolerant of desiccation and a capacity for rapid rehydration, but also by a repertoire of behavioral patterns which tend to "hold" populations in more humid microclimates (Price and Russell-Hunter, 1975; Russell-Hunter and Meadows, 1965).

Despite the discontinuities in most series, the broad ecological "rules" of littoral zonation, as elucidated by the Stephensons and by Southward (Stephenson and Stephenson, 1949, 1972; Southward, 1958b), including the prediction that no species thriving below MLWST can extend its range above MTL, hold for our snails. As often set out, environmental conditions just below MLWST are not greatly different from those in the depths of the ocean, while animals living above MHWNT are practially terrestrial. It is important to distinguish between the rather rigid vertical zonation of sessile animals such as barnacles and that of mobile animals such as our snails, where migratory behavior can modify distribution patterns on a shorter or longer time-scale. As discussed some years ago by G. E. Newell (Newell, 1954, 1958a, b; Smith and Newell, 1955; see also Newell, 1964), populations of *Littorina* spp. show both seasonal and shorter-term vertical shifts on the shore. Another significant factor concerns the considerable differences in dispersal ability which arise from differences in the eggs and larvae (see mid-columns of Figure 6). As a result of electrophoretic studies on polymorphic

esterases in New England and Canadian Atlantic populations, Berger (1973) has concluded that marked differences in such gene frequencies in the three common *Littorina* species are correlated with the extent of larval dispersal (there being significantly less local geographic differentiation in *L. littorea* than in the other two species).

To recapitulate, the patterns of aerial and aquatic respiration described here reflect vertical zonation. The patterns shown by *Littorina obtusata* differ but little from those in the three sublittoral species, though *L. obtusata* shows a greater tolerance of high temperatures and a more "balanced" aerial respiration. The case of *Littorina littorea* is of particular interest. Its great success as the dominant snail of the midlittoral depends, among other things, upon its capacity to maintain a degree of regulation in its aquatic respiration over the more usual environmental temperature range (17° C–32° C). This capacity for regulation in aquatic oxygen uptake appears to dictate the pattern of its aerial respiration (see Fig. 4), and may have prevented its developing any pattern of diapause which would be more appropriate to the greater range of air temperatures in the upper littoral. Such a pattern (involving a reversible torpor under high temperatures) *does* characterize the respiratory schesis of *Littorina saxatilis,* ensconced at higher tidal levels. Lastly, *Melampus* is primarily an air-breather with only a limited capacity for aquatic respiration at higher temperatures (McMahon and Russell-Hunter, 1975).

As discussed elsewhere (McMahon and Russell-Hunter, 1974, 1977), interspecific differences in these snails in responses to low oxygen stress are less related to vertical zonation, but rather to microenvironmental features and to the physiological ecology of each individual species. In contrast, in these temperature relations of oxygen consumption, interspecific—and even some infraspecific—differences are more directly related to degrees of submergence/exposure, and hence to the vertical distribution patterns of the snails on the seashore.

In addition, one of us (W.D.R-H.) wishes to take this opportunity to recall with gratitude his own first introduction by two different biologists (three decades ago at Millport in Scotland) to the fascination of intertidal snails. My field introduction to littoral zonation was by Richard Elmhirst, an extraordinary naturalist who published little but freely communicated much of his extensive knowledge of the living animals of the shore, and who died in 1948; while my introduction to the physiological implications of littoral life, particularly among snails, came from C. M. Yonge (now Sir Maurice Yonge) who is still, happily, engaged in his worldwide studies elucidating function and form in marine molluscs. They both contributed much to the background of this and other papers.

Summary

Aerial and aquatic rates of oxygen consumption were determined over a range of 5° to 45° C at 5° C intervals for six species of marine littoral snails: including

the sublittoral species, *Acmaea testudinalis, Mitrella lunata,* and *Lacuna vincta;* and the truly intertidal species, *Littorina obtusata, L. littorea,* and *L. saxatilis.* Polarographic oxygen electrodes were used with normally active snails collected from populations on Nobska and Manomet Points, Massachusetts.

Three subtidal species, *A. testudinalis, Lacuna vincta,* and *M. lunata,* do not display any metabolic adjustment to increasing temperature, with thermal limits reached at 30° to 35° C. Aerial respiration in *A. testudinalis* is similar to aquatic O_2 uptake, but rates average only 36.4% of aquatic rates.

The intertidal congeners, *Littorina obtusata, L. littorea* and *L. saxatilis,* have varying degrees of aerial and aquatic metabolic regulation with increasing temperature. *L. obtusata,* a low intertidal snail exposed to air for 15% to 45% of the tidal cycle, displays a respiratory pattern of "passive endurance" to high temperatures both in air and in water. *L. littorea,* the dominant snail of the mid-littoral region, remains active when exposed to air (30% to 75% of the tidal cycle) and has a zone of metabolic regulation between 20° C and 30° C. Over this, the normal ambient temperature range, the Q_{10} closely approximates one, and nearly equivalent O_2 uptake rates occur in air and in water. *L. saxatilis* from the upper littoral region is exposed to air for 70% to 95% of the tidal cycle and is characterized by reduced aerial and aquatic O_2 uptake rates above 25° C, representing a reversible torpor up to its thermal maximum at 44° C.

For these six snail species, respiratory responses to increasing temperature are thus directly related to the pattern of vertical distribution in the intertidal environment. Discussion of this relationship stresses that the evolution of other near-terrestrial structures and functions in littoral snails has proceeded in a discontinuous fashion. Despite this, the temperature responses in respiration parallel the functional morphology of the pallial structures and the physiological patterns of response to low oxygen stress, as well as adaptive features of reproduction, larval development, water-control, and nitrogenous excretion.

NOTE ADDED IN PRESS

Difficulties in the systematic status of one of our six species, *Littorina saxatilis,* have long been appreciated, but it has usually been regarded as constituting a single but polymorphic species. While both the present paper and another on the effects of low oxygen stress on the respiration of littoral snails (McMahon and Russell-Hunter, 1977) were being revised for publication, two important contributions on *Littorina* spp., one British (Heller, 1975) and one Russian (Mileikovsky, 1975), became available. On the basis of a variety of evidence, including different allozyme patterns for esterases from disc electrophoreses and differences in penial anatomy, Heller (1975) has concluded that his natural populations of the "*saxatilis*" aggregate in Wales consist of four separate but essentially sympatric species. Whatever is subsequently elucidated regarding the taxonomy of the species currently called *Littorina saxatilis* on the Atlantic coast of the United States, we had safeguarded the identification of the material used in both of our respiratory studies by depositing (while the work was still in progress, 1973–74) voucher specimens of our "*L. saxatilis*" in the collections established by Professor R. Tucker Abbott for this purpose at the Delaware Museum of Natural History. Both Heller (1975) and Mileikovsky (1975) also discuss evidence that certain forms in the "*saxatilis*" aggregate are viviparous and others oviparous. After a survey of 39 species of littorinids throughout the world, Mileikovsky concludes that any oversimple scheme

representing these snails as "text-book" examples of a supposed obligate relationship between pattern of larval development and intertidal zonation "must be rejected." If future work should show that the populations currently termed *Littorina saxatilis* on the U. S. Atlantic coast include both viviparous and oviparous forms, the necessary modification of the reproductive data summarized here need not alter the general conclusion (see Figure 6, and discussion on above) that the evolution of near-terrestrial structures and functions in littoral snails has proceeded in a discontinuous fashion.

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